PATENT
P-5808/1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Applicant(s)	Dimitrios Manoussakis		
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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF DIMITRIOS MANOUSSAKIS UNDER 37 C.F.R. § 1.132

Sir:

I, DIMITRIOS MANOUSSAKIS, do hereby declare and state:

1. I am a co-inventor of the subject matter disclosed and claimed in the above-identified patent application (herein referred to as the present application).

2. I am currently a Principal Engineer, in the Preanalytical Systems business unit at Becton, Dickinson and Company, Inc. which is the assignee of the present application.

3. I received a Bachelors Degree in Physics and Mathematics from New York University; and a Masters Degree in Mechanical Engineering from SUNY at Stonybrook. I previously held the positions of Project Engineer also in the Preanalytical Systems. I have at least 10 years experience in the field of biological sample collection, including blood, and am familiar with, blood collection tubes and blood separation technologies including gel separation.

4. I have reviewed the currently pending claims of the present application. I have also reviewed US Patent No. 4,350,593 to Kessler (herein referred to as "Kessler") and US Patent No: 3,997,442 to Gigliello at al. (herein referred to as "Gigliello").

5. It is my opinion that one of ordinary skill in the art, after reading Kessler and Gigliello, would understand that at the time of invention, the shape or geometry of a gel separator was designed to cause gel movement to occur at the earliest possible stages of centrifugation in order to form a barrier as soon as possible during centrifugation. In contrast, the gel geometry as claimed in my present application causes slower gel movement during centrifugation and therefore a longer time to form a barrier.

6. My co-inventors and I, have discovered that the pre-centrifugation gel geometries of Kessler and Gigliello provide undesirable results; such as poor barrier properties, sample entrapment in the barrier, premature barrier formation and the gel overshooting the final equilibrium separating position before settling back to the final equilibrium separating position, during centrifugation before separation of the sample has been completed.

7. I performed an experimental comparison between the gel geometry as claimed in the current patent application and the gel geometry of Kessler and Gigliello, in December 2006. A number of BD Serum Separation Tubes (SST) were used for the study. The gel bias angle for half of these tubes was modified to represent the "extreme" bias gel angle shown in Kessler and Gigliello (hereinafter referred to as "Kessler Tube"), as shown in Fig.1 below. The remaining SST's contained the gel geometry as claimed (e.g. claim 14) in our current patent application (hereinafter referred to as "Manoussakis Tube") as shown in Fig.1 below.

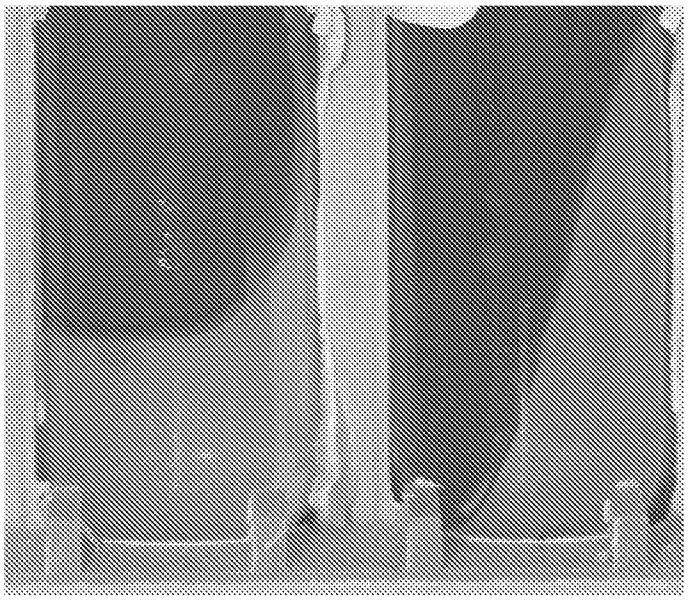

Figure 1

Manoussakis Tube **Kessler Tube**

Manoussakis Tube = BD Serum Separation Tube having Standard Bias Gel Angle.
Kessler Tube = BD Serum Separation Tube having Extreme Bias Gel Angle.

8. A human blood sample was collected into an additive free evacuated blood collection tube; a portion (8 ml) of this blood sample was then transferred into either a Manoussakis tube or a Kessler tube via the vacuum of that tube. Each tube was then inverted five times in order to promote clotting of the blood. The tube was then allowed to sit for 30 minutes, after which time the tube was then centrifuged for 10 minutes, at one of a number of centrifugal forces (1100 x g, 900 x g and 800 x g). The tube was then recovered from the centrifuge and the separated sample evaluated for Gel Smear, Gel Barrier Formation, and Blood Entrapment within Gel Barrier. The results of these tests are shown in Table 1 below.

9. The performance of the gel layer as a separator was evaluated in terms of the following properties:

Gel Smear; the lateral distance (in millimeters) that the gel layer "overshoots" the final equilibrium separating position before settling back to the final equilibrium separating position thereby leaving a smear of gel on the inside surface of the tube above the upper surface of the gel layer.

Gel Barrier Formation; a qualitative evaluation (on a scale of 1 (worst) to 10 (best)) of the integrity and smoothness of the upper surface of the gel layer after centrifugation.

Sample Entrapment within Gel Layer; a qualitative evaluation (on a scale of 1 (worst) to 10 (best)) of the degree of entrapment of whole blood or blood components within the gel layer after centrifugation.

Table 1

Centrifugal Force (units x g)	**Sample**	**Figure No**	**Gel Smear (units mm)**	**Gel Barrier Formation**	**Sample Entrapment within Gel Layer**
1100	Kessler	2 & 3	9 to 10	7	7
	Manoussakis	2 & 3	7 to 8	10	9
900	Kessler	2 & 4	8	6	7
	Manoussakis	2 & 4	8	9	10
800	Kessler	2 & 5	7	4	6
	Manoussakis	2 & 5	4	9	9

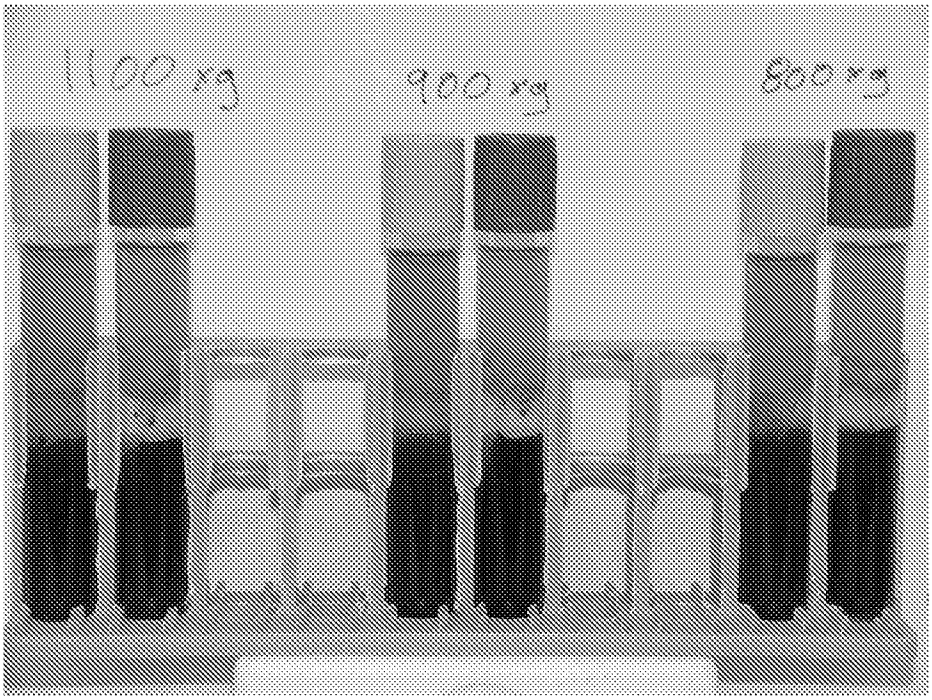

Figure 2
Tubes are shown after centrifugation
Tubes with Light Tops are Manoussakis Tubes
Tubes with Darker Tops are Kessler Tubes

Centrifugal Force 1100 x g

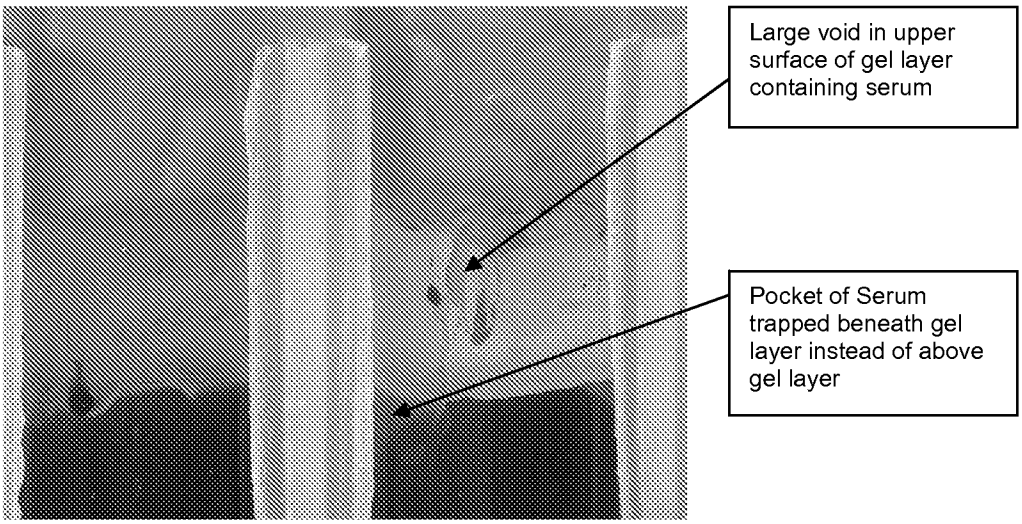

Figure 3
Manoussakis Tube Kessler Tube

Centrifugal Force 900 x g

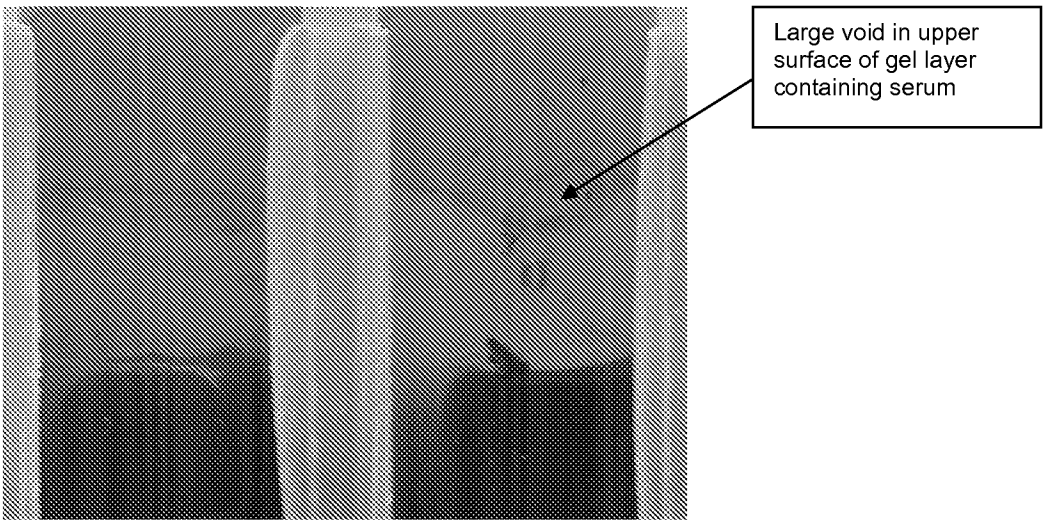

Figure 4

Manoussakis Tube Kessler Tube

Centrifugal Force 800 x g

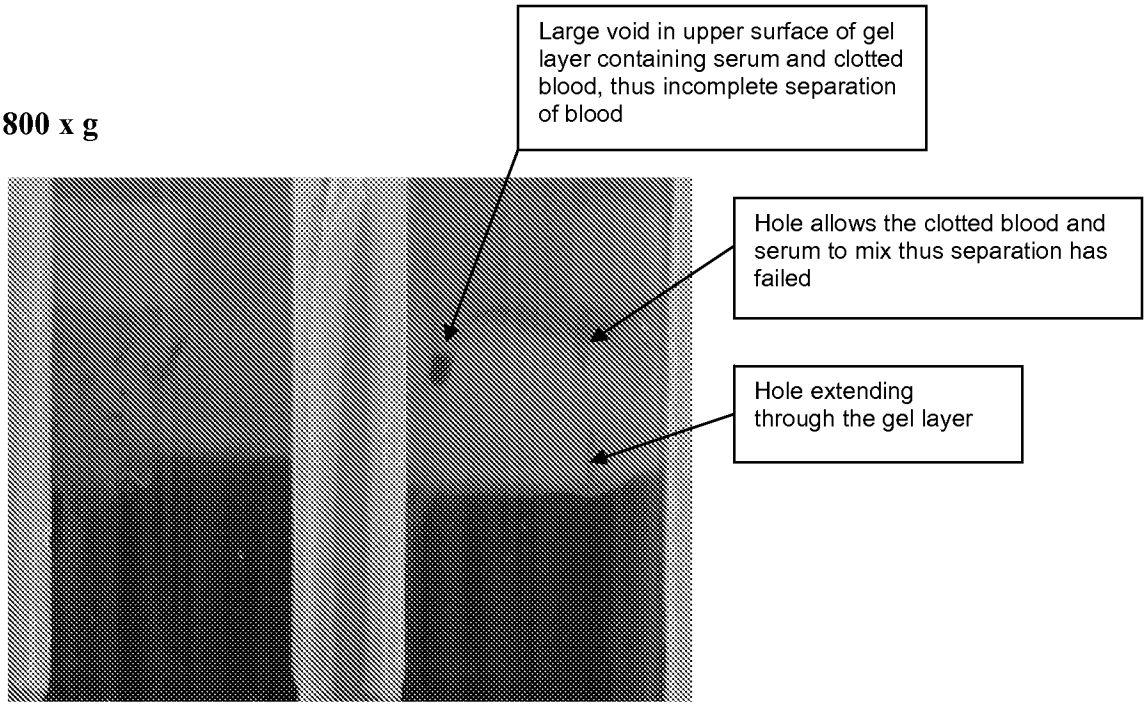

Figure 5

Manoussakis Tube Kessler Tube

10. The experimental results (Figures 2 to 5) clearly show that the initial "extreme" bias gel angle of the Kessler tube results in a poorer quality gel layer after centrifugation and in some cases incomplete sample separation, when compared to the Manoussakis tube "standard" bias gel angle, at all the centrifugal forces tested.

11. Figure 3 shows the sample after centrifugation at 1100 x g, clearly the Kessler tube has a poorer gel barrier with a large void filled with serum in the upper surface of the gel layer extending almost 66% of the thickness of the gel layer. It should also be noted that the initial Kessler gel angle resulted in a pocket of serum being trapped below instead of above the gel layer.

12. Figure 4 shows the sample after centrifugation at 900 x g, the Kessler tube once again has a poorer gel barrier with a large void filled with serum in the upper surface of the gel layer.

13. Figure 5 shows the sample after centrifugation at 800 x g, the Kessler tube appears to have produced a very poor gel barrier with a large void in the upper surface of the gel layer containing clotted blood and serum thus a barrier has been formed before complete separation of the sample. Furthermore the gel barrier appears to be incomplete, with a hole through the gel layer at the interface with the inner surface of the tube allowing the clotted blood and serum components to mix.

14. In contrast, the Manoussakis tube samples (Figs. 2 to 5) show a continuous gel layer with no large voids; holes or pockets of serum below the gel layer after centrifugation, at all the centrifugal forces test.

15. It can be concluded from this comparison that the gel geometry as claimed in the present application provides a better final gel barrier as a separation means than the "extreme" bias gel angle as shown in US Patent No. 4,350,593 to Kessler and US Patent No: 3,997,442 to Gigliello at al.

16. I declare further that all statements made in this Declaration of my own knowledge are true, and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and that like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 19 of the United States code and that such willful false statements may jeopardize the validity of this application and any patent issuing thereon.

Dated: Oct. 8/2008

D MANOUSSAKIS

Dimitrios Manoussakis